

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : James S. Norris et al. Art Unit : 1633
Serial No. : 10/082,973 Examiner : Janet L. Epps-Ford
Filed : February 26, 2002 Conf. No. : 8113
Title : TISSUE-SPECIFIC AND TARGET RNA-SPECIFIC RIBOZYMES

Mail Stop Amendment

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF JAMES S. NORRIS UNDER 37 C.F.R. § 1.131

I, James S. Norris, hereby declare as follows:

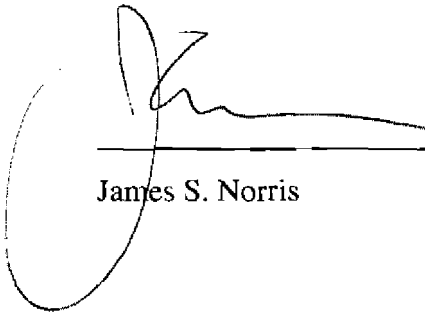
1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.
2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).
3. The Norris *et al.* reference lists its publication date as June 11, 1998.
4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, Gary A. Clawson, Michael G. Schmidt, Brian D. Hoel, Wei-Hua Pan, Joseph W. Dolan, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shani Schalles' laboratory notebook, each produced under Gary A. Clawson's supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP

sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

6/11/02
Date

60427105.doc


James S. Norris

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Alexandria, VA 22313-1450

DECLARATION OF GARY A. CLAWSON UNDER 37 C.F.R. § 1.131

I, Gary A. Clawson, hereby declare as follows:

1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.
2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).
3. The Norris *et al.* reference lists its publication date as June 11, 1998.
4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, James S. Norris, Michael G. Schmidt, Brian D. Hoel, Wei-Hua Pan, Joseph W. Dolan, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shani Schalles' laboratory notebook, each produced under my supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

Applicant : James S. Norris et al.
Serial No. : 10/082,973
Filed : February 26, 2002
Page : 2 of 2

Attorney's Docket No.: 14017-004002 / PSU 96-
1566

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

JUNE 11, 2007
Date

Gary A. Clawson
Gary A. Clawson

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : James S. Norris et al. Art Unit : 1633
Serial No. : 10/082,973 Examiner : Janet L. Epps-Ford
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Alexandria, VA 22313-1450

DECLARATION OF MICHAEL G. SCHMIDT UNDER 37 C.F.R. § 1.131

I, Michael G. Schmidt, hereby declare as follows:

1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.
2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).
3. The Norris *et al.* reference lists its publication date as June 11, 1998.
4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, James S. Norris, Gary A. Clawson, Brian D. Hoel, Wei-Hua Pan, Joseph W. Dolan, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shani Schalles' laboratory notebook, each produced under Gary A. Clawson's supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

5. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.



11 June 2007

Date

Michael G. Schmidt

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : James S. Norris et al. Art Unit : 1633
Serial No. : 10/082,973 Examiner : Janet L. Epps-Ford
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P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF BRIAN D. HOEL UNDER 37 C.F.R. § 1.131

I, Brian D. Hoel, hereby declare as follows:

1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.
2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).
3. The Norris *et al.* reference lists its publication date as June 11, 1998.
4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, James S. Norris, Michael G. Schmidt, Gary A. Clawson, Wei-Hua Pan, Joseph W. Dolan, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shani Schalles' laboratory notebook, each produced under Gary A. Clawson's supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

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June 10 '07

Date

Brian D. Hoel

Brian D. Hoel

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : James S. Norris et al. Art Unit : 1633
Serial No. : 10/082,973 Examiner : Janet L. Epps-Ford
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Alexandria, VA 22313-1450

DECLARATION OF WEI-HUA PAN UNDER 37 C.F.R. § 1.131

I, Wei-Hua Pan, hereby declare as follows:

1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.

2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).

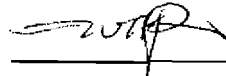
3. The Norris *et al.* reference lists its publication date as June 11, 1998.

4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, James S. Norris, Michael G. Schmidt, Brian D. Hoel, Gary A. Clawson, Joseph W. Dolan, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shani Schalles' laboratory notebook, each produced under Gary A. Clawson's supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

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6-11-07

Date



Wei-Hua Pan

Jun 13 2007 8:16PM HP LASERJET FRX

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MICRO

PAGE 02/03

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : James S. Norris et al. Art Unit : 1633
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Alexandria, VA 22313-1450

DECLARATION OF JOSEPH W. DOLAN UNDER 37 C.F.R. § 1.131

I, Joseph W. Dolan, hereby declare as follows:

1. I am an inventor of currently pending claims 39-55 of the above-referenced patent application.

2. In an Office Action dated January 11, 2007, claims 39, 43-48, 50-51, and 53-55 were rejected under 35 U.S.C. § 102(a) as allegedly being anticipated by Norris *et al.* (WO98/24925).

3. The Norris *et al.* reference lists its publication date as June 11, 1998.

4. Prior to June 11, 1998, and thus necessarily before the publication date of the Norris *et al.* reference, James S. Norris, Michael G. Schmidt, Brian D. Hoel, Wei-Hua Pan, Gary A. Clawson, and I worked together in this country to complete the conception of the invention recited in present claims 39, 43-48, 50-51, and 53-55 of the above-referenced patent application, and to reduce said invention to practice, as evidenced by a copy of pages from Ping Xin's laboratory notebook and pages from Shami Schalles' laboratory notebook, each produced under Gary A. Clawson's supervision, which is attached as Exhibit A. The pages include multiple examples of pCHOP sequences. The dates on these pages, all of which are prior to June 11, 1998, have been blacked out.

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JUN 13 2007 8:16PM HP LASERJET FAX

p.3

06/11/2007 14:39 7924882

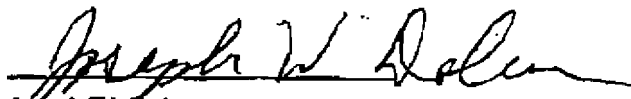
MICRO

PAGE 03/03

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6/13/07

Date


Joseph W. Dolan

60427107.doc

Page 1 of 3
9:42 AM

```
File: 19-GC383
Sample: GC383
Comment:
Lane Number: 19
Channel Number: 100
Number of Scans: 11812
Length: 898
Run started at: [REDACTED] 12:49
Run stopped at: [REDACTED] 22:49
Gel: Gel File
Dyeset/Primer: DT4Ac(A Set-AnyPrimer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A
```

Data Analysis

```
Base Call Start: 1323
Base Call End: 11812
Primer Peak Loc.:1323
Signal: G (354), A (393), T (181), C (274)
Matrix Name: Rhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.29 - ABI100
```

[illegible]



Model 377
Version 2.1.1

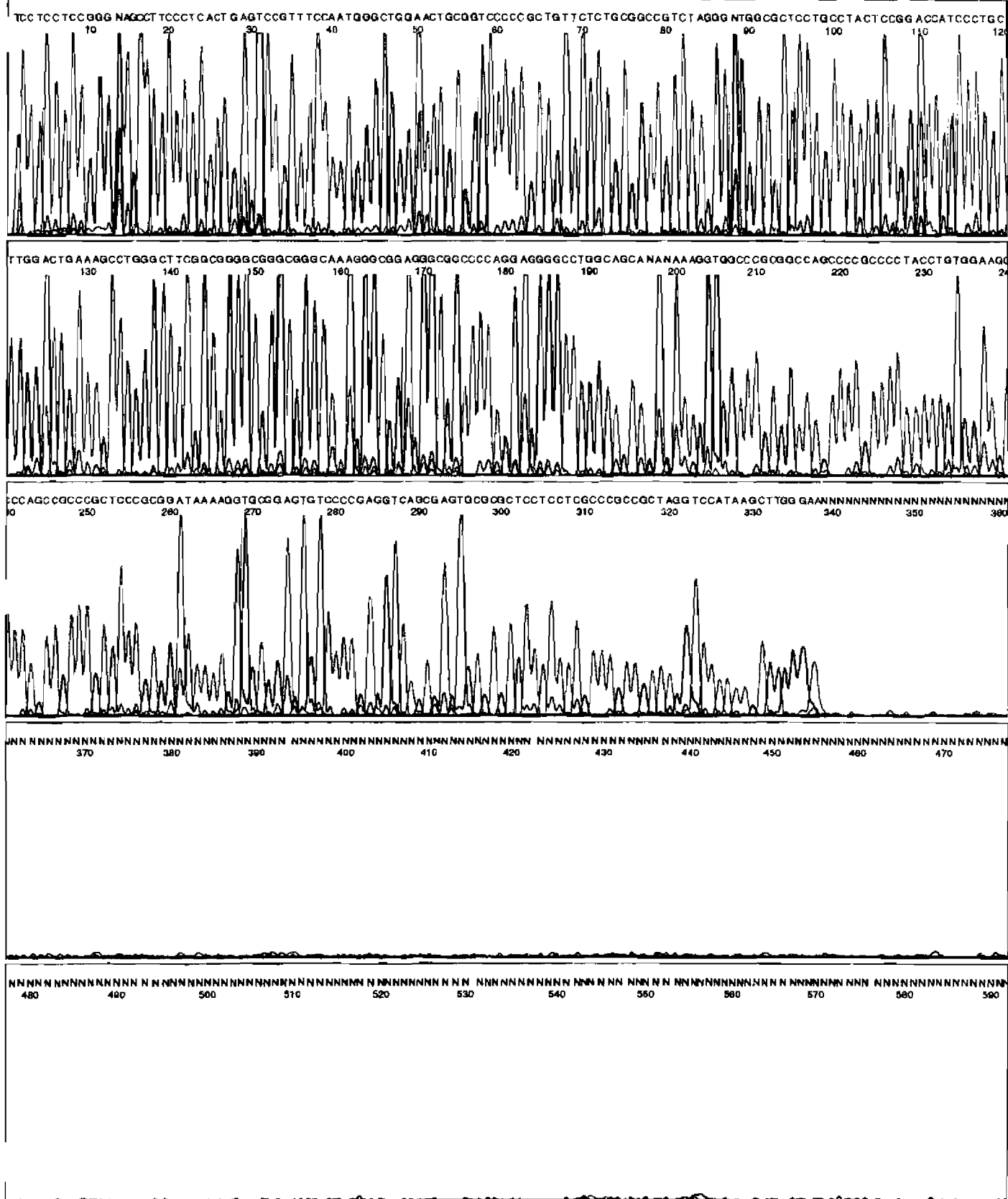
19-GC383

GC383
Lane 19

Signal G:354 A:393 T:181 C:274
DT4%Ac(A Set-AnyPrimer)
Rhodamine
Points 1323 to 11812 Base 1: 1323

Page 2 of 3

Spacing: 12.29 ABI100



Spacing: 12.29 A8/100

[illegible][illegible]

NNNNNNNNNNNNNNNNNNNNNNNN N NNNNNNN NNNNN NNNNNNNNNNNNNNNNNNNNNNNNN NN NNNNN NNNNNNNNNNNNN
20 830 840 850 860 870 880 890

K7 promoter — Continue S.S.

PCR K7 promoter with Pfu DNA Polymerase

10 μ l 10x buffer

8 μ l 2.5 mM dNTPS

2 μ l template DNA, 100 ng/ μ l
primer 1 μ l : 100 pmol

Claw 429

Claw 430

Pfu. 2 μ l.

H₂O 68 μ l.

to two tubes.

94°C. 45"

94°C 45"

66°C 45"

72°C 1 min

28 Cycles.

No PCR product!

Change annealing T_m:

57°C. 61°C. 30 Cycles.

Mix half of the first time volume. — 2 x 25 μ l

Try 57°C and 61°C Annealing. 28 cycles
other condition same. Nothing!

Intro. / K α / pCMV β .

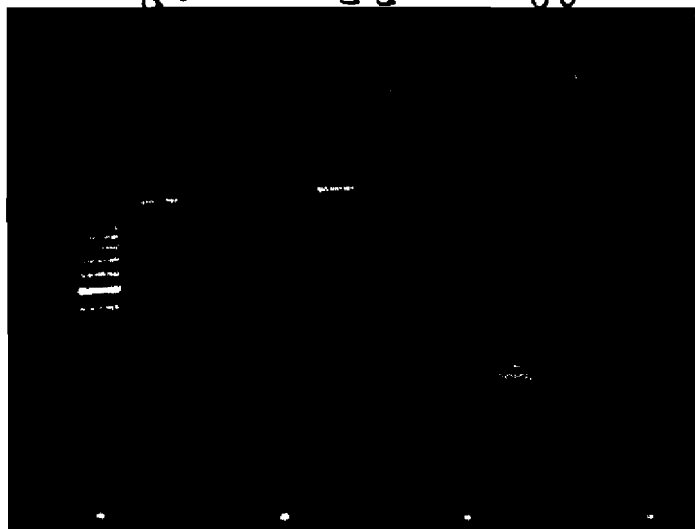
① Cut E/K α -film pCX-GFP-1 with SalI + HincII.
Isolate ~ 700bp band, blunt.

② Cut pCMV β with EcoRI + XhoI blunt.

Ligate ① + ②.

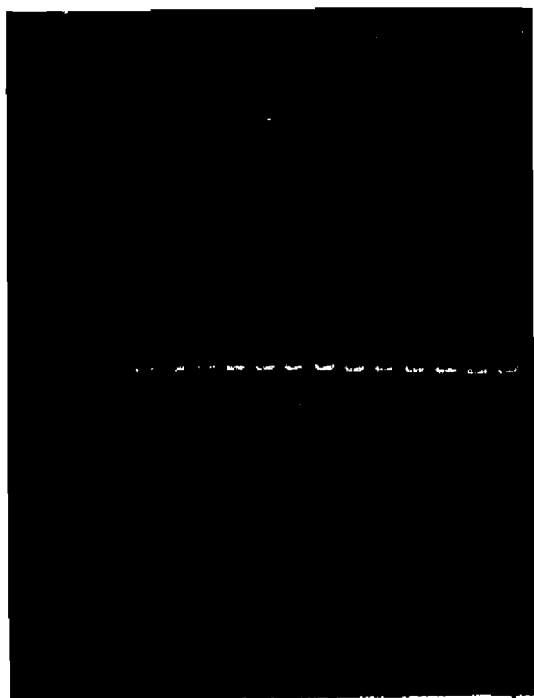
Check orientation; cut with HindIII



	1	2	3	4	5	6	7	8	9
1	Do PCR again.								
2									
3	Block 2.				Block 3		Block 1		
4	52°C annealing.				56°C		60°C		
5	# 1. 2. 3.				# 4. 5. 6.		# 7. 8. 9.		
6									
7	1, 4, 7. Tag.				Tag buffer				
8									
9	2, 5, 8. pfu.				pfu buffer.				
10									
11	3, 6, 9. pfu.				Tag buffer.				
12									
13					52		55		60
14	PCR Program:								
15	94°C. 2min.								
16	94°C. 45"								
17	Annealing. 45"								
18	72°C. 1'20"								
19	28 Cycles.								
20									
21									
22									
23									
24									
25	Mix as before.				Right product should be ~380				
26	PT, EtOH ↓ (2+5).								
27									
28	+ 41 μl H ₂ O; 1 μl GC432 (pIND/chop).								
29	+ 2.5 μl Buffer 2; 2.5 μl Buffer 4.								
30	1.5 μl pme. 1.5 μl Hind III. 37°C. 1-15 — 4-15.								
31									



Redo PCR. Cut together with Gc#32.
 pt. ZtoHv. ligation. Transform. PCR screen.



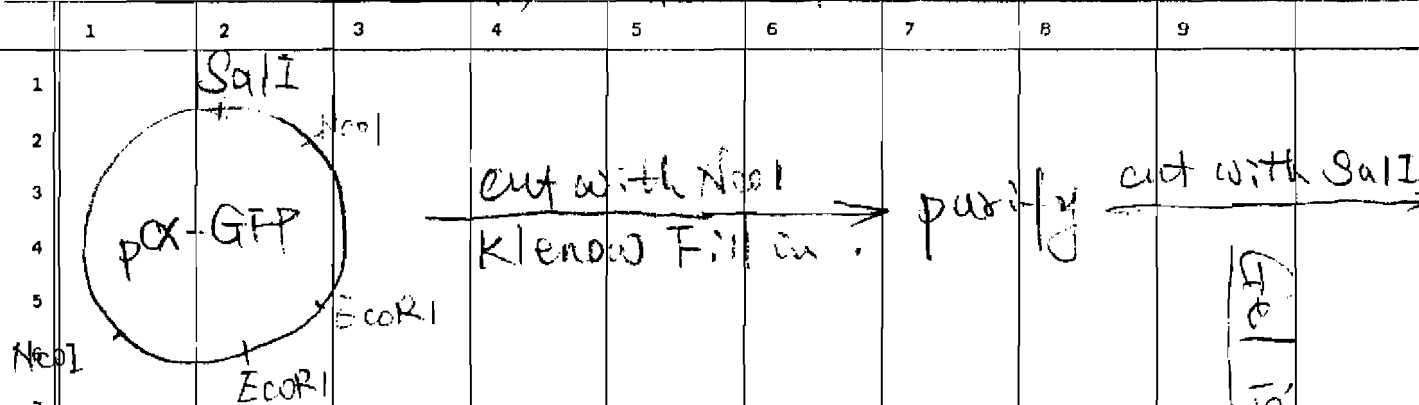
PIND/chop

PIND only

PIND/chop. PCR with Glaw 387 + 388.
 product is about 350 bp. After add K7
 promoter, PCR product should be 700 bp.

Because there are two pmeI site in
 PIND, plan to clone K7 to pmeI/HindIII.
 gap is wrong from beginning. That's why
 S's can't get it. S's design it.

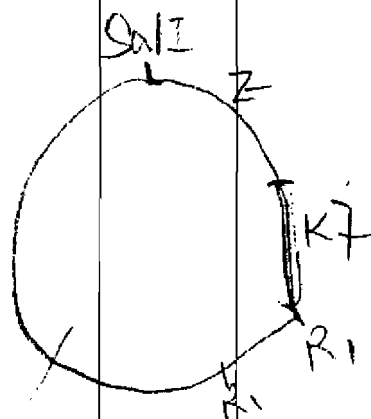
Enhancer / K7 / GFP → pCX-GFP



From K7/pND. Get K7 promoter with pmtI/EcoRI end

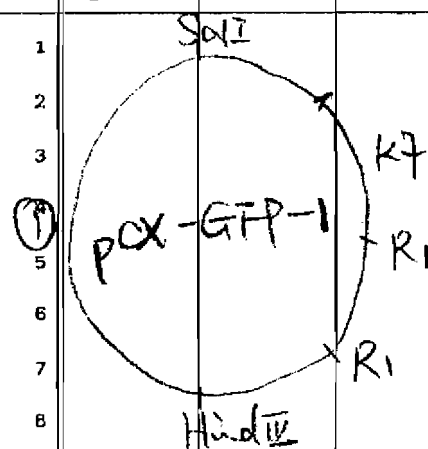
Cut pCX-GFP with SalI, partial cut EcoRI.
keep R1 frag.

Ligation:



pCX-GFP-1

Enhancer / K7 / Snip / ~~pox-GFP~~



$\xrightarrow[\text{gel.}]{\text{SalI} + \text{HindIII}}$

isolate ~700bp band
GeneClean.

② get Snip cassette with HindIII / XbaI ends.

③ cut pox-GFP, with HindIII. blunt. Ligate.
cut it again with EcoRI. Religate large band.
cut it with SalI + XbaI. isolate large band.

Ligation ① + ② + ③.



Model 377
Version 2.1.1

03-GC387

Signal G:463 A:365 T:294 C:482
DT (BD Set Any-Primer)

Page 1 of 3
9:35 AM

Data Collection

File: 03-GC387
Sample: GC387
Comment:
Lane Number: 3
Channel Number: 31
Number of Scans: 11812
Length: 904
Run started at: 15:44
Run stopped at: 01:44
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1246
Base Call End: 11812
Primer Peak Loc.: 1246
Signal: G (463), A (365), T (294), C (482)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 11.85 - ABI100

```
1 CCCAGCTTTT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTT GGAAOCCCTGA TGAGTCCGTG 70
71 AGGACGAAAC GATGACATTC TGCTGACCAG AITCACGGTC AGCAGAAITGT CATGTCGGT TOCAGGATCC 140
141 TTGCTTGAAT TCCAAGGGTC TGCGCAACGA CGACGATGAG GTACCATATC GTCTGCTGTG CGCACTGATG 210
211 AGGCGGTGAG GCGGAAACCC TTGACGGGTT CCTATGCGGC CGCTCTAGAG GGCCCAATTC GCOCTATAGT 280
281 GAGTCGTATT ACAATTCAC TGGCGTGGT TTACAACGTC GTGACTGGGA AAACCCCTGGC GTTACCCAC 350
351 TTAATCGCTT TGCAGCACAT CCCCCTTTTCG CCAGCTGGCG TAATAGCGAA GAGGCCCGCA CCGATCGCCC 420
421 TTTCCAACAG TTGCGCAGOC TGAATGGCGA ATGGAACGCG CCTGTAGCGG CGCATTAAGC GCGCGGGGTG 490
491 TGGTGGTAC GCGCAGGTG ACCGCTACAC TTGCCAGGCG CCTAGCGGCC GCTCCTTTTCG CTTTCTTCCC 560
561 TTCTTTTTC GGCAGTTTC CCGGCTTTC CCGTCAAGCT CTAAATCGGG GGCTOCCCTT AGGGTTCGGA 630
631 TTTAATGCTT TACGGGACCT TCGACCCCAA AAAACTTGAT TAGGGTGATG GTTCACGTAA TGGGCCATTG 700
701 NCCGATAGA CGGTTTTTCG CCGTTTGAAG TTTGGAAGTC CACGTTCTTT AATAAGTGA CTCFTGTTCC 770
771 AAACGTGAAC AACCCTTAA CCGTTATCTT GGGGCTATTC NTTTGGATTT TATNANGGA TTTTGCCCGA 840
841 TTTTNGGCC TATTTGGGT AAAAATGA ANCTGGNTT TAACCAAAAA TTTTAACCGC GNAA 910
```

Chop/size Right: Save

Class 5.



Model 377
Version 2.1.1

26-GC446

Signal G:149 A:117 T:103 C:116
DT {BD Set Any-Primer}

Page 1 of 3
8:46 AM

Data Collection

File: 26-GC446
Sample: GC446
Comment:
Lane Number: 26
Channel Number: 128
Number of Scans: 12992
Length: 1057
Run started at: 16:55
Run stopped at: 03:56
Gel: Gel File
Dyeset/Primer: DT {BD Set Any-Primer}
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1362
Base Call End: 12992
Primer Peak Loc.: 1362
Signal: G (149), A (117), T (103), C (116)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.23 - ABI100

1 GACGGCCAGT GAATGTGAAT ACGACTCACT ATAGGGCGAA TTGGGCCCCC TAGAGCGGCC GCATAGGAAC 70
71 GCGTCAAGGG TTTCGGCCCTC ACGGCCTCAT CAGTGGCGAA CGACGACGAT GTGGTACCTC ATCGTCGTCTG 140
141 TTCCGCACAC CCTTGGAAAT CAGGCAAGGA TCCGTGAACC CACGATGACA TTCTGCTGAC CGTGAATCTG 210
211 GTCAGCAGAA TGTGATCGTT TGTCTCTCAC GGACTCATCA GGGTTCCAAA GCTTGATGCA TAGCTTGAGT 280
281 ATTCTATAGT GTCACCTAAA TAGCTTGGCG TAATCATGGT CATAGCTGTT TCTGTGTGA AATTGTTATC 350
351 CGCTCACAAT TCCACACAAC ATACGAGCCG GAAGCATAAA GTGTAAAGCC TGGGGTGGCT AATGAGTGAG 420
421 CTAACACACA TTAATTGGGT TGGCTCACT GCGCGCTTC CAGTGGGAA ACGTGTGTG CCAGCTGCAT 490
491 TATGAATCG GCCAACCGCG GGGGAGAGGC GGTTCGGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG 560
561 ACTCGCTCGG CTGGGTGTT CCGCTCGGC GAGCGGTATC AGCTCACTCA AAGCGGTAA TACCGTTAT 630
631 CCACAGAATC AGGGGGATAA CGCAAGGAAA GAACATGTA AGCAAAAAG CCAAGCCAAA AGGCCAGGA 700
701 ACGTAAAAA AGGCCCGGT TGTCTGGGT TTTCCTATN GNTCCGNC CCGTTGGAAG AAGCATACAA 770
771 AAAAATCGAC GCTTAAAGTC AANAAGGTG GCGAAAACC CCGACAAGGA CTCTTTTAAA GATACCCAAG 840
841 GCGTTTTC CCGCTTGGG AAAGCTTCC CTCTTGGCG CTCTTCTCTT GGTTCGGA ACCCTTGGCC 910
911 CGGTTTAAAC CGGATTACC CTGGTCCCG GNTTCTTC CTTTTTGGG AAAGCTTTC GGTGCTTTT 980
981 CTCTTATAG NNTAACGCT TGTAAAGGNN ATTCTTATAT TTGGGNGTT AAGTCCGTT CNGTCTTCA 1050
1051 AANCTCC 1120

dG - chop / PCR = Right.

Clow 104. Sure:

#9



Model 377
Version 2.1.1

19-GC432

Signal G:134 A:105 T:95 C:106
DT (BD Set Any-Primer)

Page 1 of 3
9:02 AM

Data Collection

File: 19-GC432
Sample: GC432
Comment:
Lane Number: 19
Channel Number: 99
Number of Scans: 12992
Length: 1045
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1304
Base Call End: 12992
Primer Peak Loc.: 1304
Signal: G (134), A (105), T (95), C (106)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.06 - ABI100

Hint

1	GAGAAAGAAG AACUCACACA CAGCTAGCGT TTAAACTTAA GCTTTTGGAAAC CCGTATGAGT CCGTGAGGAC	70
71	GAAACGATGA CATTCCTGCTG ACCAGATTCA CCGTCAGCAG AATGTCATCG TCGGTTCACAG GATCCTTGCC	140
141	TGAATTCCAA GGGTCTGGGC AACGACGACG ATGAGGTACC ACATCGTCGT CGTTGCCAC TGATGAGGCC	210
211	GTGAGGCCGA AACCTTTGAC GCGTCTCTAT GCGGCCGCTC TAGAGGGCCC GTTTAAACCC GCTGATCAGC	280
281	CTGACTGTG CTTCTAGTT GCCAGCCATC TGTGTGTTC CCGTCCCCCG TGGCTTCCTT GACCTTGAA	350
351	GGTGCCACTC CCACTGTCTT TTCTTAATAA AATGAGGAAA TTGCATCGCA TTGCTGAGT AGGTGTCATT	420
421	CTATTCTGGG GGGTGGGGTG GGGCAGGACA GCAAGGGGGA GGATTTGGAA GACANNNNN NGNNTGCTGG	490
491	GGATGCNNGN GGGVINTATG GNTTNTGAGG CNGAAAGAA CCANITGGGN TTNGGGGGN NINCNNACNN	560
561	GNNCTGNNNN GGNCCNNNA GNGCGGNGG NNGNNGGN NNGNNGCAGN GNGCNCNTN NNTNGGNN	630
631	GNGNNNTAG NGNNGNINN TNNNGNNNN NNNTNNNNC TNNNGNNN NGNNGNNNG GNTTNNNN	700
701	GNNNGGNNN NNNNNNNNG NGGNNNNNN TNNNGGNNN NNNNNNNNN NGNNNNNNN NNNNNNNNN	770
771	NNNNNNNNN NNNNNNNNN NGNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN	840
841	NNNNNNNNN TNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN CNNNNNNNN	910
911	NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN	980
981	NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNCC	1050

pIND / chop . Right !

Save !



Model 377
Version 2.1.1

17-GC430

Signal G:275 A:261 T:228 C:256
DT (BD Set Any-Primer)

Page 1 of 3
9:01 AM

Data Collection

File: 17-GC430
Sample: GC430
Comment:
Lane Number: 17
Channel Number: 90
Number of Scans: 12992
Length: 1057
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1267
Base Call End: 12992
Primer Peak Loc.: 1267
Signal: G (275), A (261), T (228), C (256)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 11.93 - ABI100

```
1  CCCACCTTTT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTT GGAACCCCTGA TGAGTCCGTG 70
71  AGGACGAAC GATGACATTC TCGTGACCAG ATTACGGTTC AGCAGAATGT CATGTGCGGT TCCAGGATCC 140
141 TTCAAAGACT AATGAGTCCG TGAGGACGAG ACGAGGATCG AATTCCAAAG GTCTGCGCAA CGACGACGAT 210
211 GAGGTACACC ATCTGTCTCG TTGCGCACTG ATGAGGCGGT GAGGCCGAAA CCGTIGACGC GTTCTTATGC 280
281 GCGCGCTCTA GAGGGGCCAA TTGCGCCTAT AGTGAGTCGT ATTACAATTC ACTGGCCGTC GTTTTACAAC 350
351 GTCGTGACTG GGAACCCCTT GCGGTACCC AACTTATTCG CCGTGCAGCA CATCCCCCTT TCGCCAGCTG 420
421 GCGTAATAGC GAAGAGGCCG GCACCGATCG CCGTTCCTCA CAGTTGCGCA GCGTGAATGG CGAATGGACG 490
491 CGCCCTGTAG CCGCGCATTA AGCGCGGCGG GTGTGGTGGT TACGCGCAGC GTGACCGCTA CACTTGCCAG 560
561 CGCCCTAGCG CCGCTCTCTT TCGCTTTCTT CCGTCTCTTT CTGCGCAGT TCGCCGGCTT TCCCCGTCAA 630
631 AGCTCTAAAT CCGGGGCTTC CCGTTAGGGT TCCGATTAG TCGTTTACGG CAGCTCGACC CCAAAAAACT 700
701 TGATTAGGGT GATGGTTCAC GTAGTGGGCC ATNGGCCCTG ATAGACGGTT TTTCGGCCCT TTGACGTGG 770
771 AAGINCAGT TTCTTTTAAAT AAGTGGGACT TCTTGGTTC CAAAACCTTG GAACCAACCA CTTTAAACCC 840
841 TTATNTTGG GCGCTATTC TTTTGGATT TAATTAAAGG GAATTTTGG CCGAATTTCT GGNCGTINTT 910
911 GGGTTNAAA AAAATGGAGC TTGGATTTTA ANCAAAAATT TTAAACCGCG NAAATTTTTA ANCCAAAANT 980
981 TTAAGGGGGC NCCAAGGGGG CTTCCTTNA NGGAAAACC GGAACCCCG TTGAAAAGG CCGANTNCCN 1050
1051 CAAAAAA 1120
```

PCR II / chop - pol I - in. Right!



Model 377
Version 2.1.1

07•GC420

Signal G:259 A:240 T:213 C:240
DT (BD Set Any-Primer)

Page 1 of 3
8:59 AM

Data Collection

File: 07•GC420
Sample: GC420
Comment:
Lane Number: 7
Channel Number: 45
Number of Scans: 12992
Length: 1066
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1265
Base Call End: 12992
Primer Peak Loc.: 1265
Signal: G (259), A (240), T (213), C (240)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.01 - ABI100

```
1  CGCCAGCTAT TTAGGTGACA CTATAGAATA CTCAAGCTAT GCATCAAGCT TTGGAACCCCT GATGAGTCCG 70
71  TGAGGACGAA ACGATGACAT TCTGCTGACC AGATTCAOGG TCAGCAGAAT GTCATCGTUG GTTCCAGGAT 140
141 CCTGCTCTTC TAATGAGTCC GTGAGGACGA GACCGCTGA GAATTCOAAG GGCTCCGCA ACGACGACGA 210
211 TGAGGTACCA CATCGTCGTC GTTGGCGACT GATGAGSCCG TGAGGCCGAA ACCCTTGACG CGTTCCTATG 280
281 CGCCCGCTCT AGAGGGCCCA ATTGCCCCA TAGTGAGTGC TATTACAATT CACTGGCCGT CGTTTACAA 350
351 CGTGTGACT GGGAAAACCC TGGCGTTACC CAACHTAATC GCTTGCAGC ACATCCCCCT TTGCGCAGCT 420
421 GCGTAAATAG CGAAGAGGCC CGCACCGATC GCGCTTCCCA ACAGTTGCGC AGCCIGAATG GCGAATGGAC 490
491 GCGCCCTGTA GCGCGCAAT AAGCGCGCG GGTGTGGTGG TTACGCGCAG CGTGACCGCT ACACHTGCCA 560
561 GCGCCCTAGC GCGCGCTCTT TTGCTTTCT TCCCTTCTT TCTGCGCAG TTGCGCGCT TTCCCGTCA 630
631 AGCTCTAAAT CCGGGGCTCC CTTTAGGGTT CCGATTAAAT GCTTTACGG ACCCTGACCC CAAAAAACT 700
701 TTGATTAGGG GTGATGGTTC ACGTAAGTGG GNCATTTGCC CTGATAGACG GTTTTTTCGG CCGTTTGACG 770
771 TTGGAAGTCC ACCGTTCTTT TAATAGTGG CTTCTGGTTC CNACTTGG AACCAACT TTAACCCCT 840
841 ATTTTNGGC CTATTTONT TNGAATTAT TNANGGAAT TTTTGNCCA TTTTTCGGG CCGTATNGG 910
911 GTTNAAAAAA ATGGAACCTN GATTTTAAAC CAAAAAANT TNAACCGCG AAATTTTTTA NCCAAAAAT 980
981 TCAAGGGGCG NCCAANGGCG NTTGGTTTAA AGGGGAAACC GGGGAAACC CGTTTNAAAA AGGCCCAAT 1050
1051 CCCCNNAAAA AAACCG 1120
```

PCR II / Chop - B2 - m. Right!



Model 377
Version 2.1.1

09*GC422

Signal G:231 A:216 T:193 C:223
DT (BD Set Any-Primer)

Page 1 of 3
9:00 AM

Data Collection

File: 09*GC422
Sample: GC422
Comment:
Lane Number: 9
Channel Number: 53
Number of Scans: 12992
Length: 1069
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1265
Base Call End: 12992
Primer Peak Loc.: 1265
Signal: G (231), A (216), T (193), C (223)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.01 - ABI100

```
1  CGCCAGCTAT TTAGGTGACA CTATAGAATA CTCGAAGCTAT GCATCAAGCT TTGGAACCTT GATGAGTCCG 70
71  TGAGGACGAA ACGATGACAT TCTGCTGACC AGATTACAGG TCAGCAGAAT GTCATCGTGG GTTCCAGGAT 140
141 CCTCGAAGCT GTCTGATGAG TCCGTGAGGA CGAAACCGCG TTGAGAATTG CAAGGGTCTG CGCAACGACG 210
211 ACGATGAGGT ACCACATGTT CGTGTGTGG CACTGATGAG GCGGTGAGGC CGAAACCTTT GACGGGTTCG 280
281 TATGGGGCGG CTCTAGAGGG CCGAATTGCG CCTATAGTGA GTCTATTAC AATTCACTGG CCGTCTGTTT 350
351 ACAACGTGTT GACTGGGAAA ACCCTGGGGT TACCCACTT AATCGCTTG CAGCACATCC CCTTTTCGCC 420
421 AGCTGGGGTA ATAGCGAAGA GCGCCGCAAC GATCGCCCTT CCGAACAGTT GCGCAGCCTG AATGGCGAAT 490
491 GGACGCGGCC TGTAGCGCGG CATTAAGCGC GCGGGGTGTG GTGGTTACGC GCAGCGTACG CGCTACACTT 560
561 GCCAGCGGCC TAGCGCGCGC TCCCTTCGCT TCTCTCCCTT CCTTCTCGC CACGTTCCGC GGCTTTTCCC 630
631 GTCAAAGCTC TAAATCGGGG GCTCCCTTTA NGGTTCGGAT TTAATGCTTT ACGGNACCTT GACCCCAAAA 700
701 AACTTGATTA GGGTGTGGG TTCACGTTAG TGGGCCATCG CCTGATAGA CCGTTTTCG GCTTTTTCG 770
771 GTTTGGAAGT CCACGTTCTT TTAATAGTGG GACTCTTGGT TCACAAACTT GCGAACCAAC CACTTTAAAC 840
841 CCTTATTTT TNGGGCTAAT TCCCTTTTGG AATTATTTAA NGGGAATTTT TGGCCGATTT TONGGGCTA 910
911 TTTGGGGTAA AAAAAAATGG AAGCTTGGAT TTTTAANCA AAAAAATTTT TAACCGCGCA AATTTTAA 980
981 CCCAAAAATT TCAAGGGGCC CCNAAGGGC NITGNITTA AGGGAACCC CTTTNNAAA 1050
1051 AGGCCAGTT NCCCNANT 1120
```

PCR/Chop - C3

Right:



Model 377
Version 2.1.1

10-GC423

Signal G:219 A:202 T:185 C:202
DT (BD Set Any-Primer)

Page 1 of 3
9:00 AM

Data Collection

File: 10-GC423
Sample: GC423
Comment:
Lane Number: 10
Channel Number: 58
Number of Scans: 12992
Length: 1058
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1292
Base Call End: 12992
Primer Peak Loc.: 1292
Signal: G (219), A (202), T (185), C (202)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.02 - ABI100

```
1  CCAGCTATTT AGGTGACACT ATAGAATACT CAAGCTATGC ATCAAGCTTTT GGAACCCCTGA TGAGTCCGTG 70
71  AGGACGAAAC GATGACATTC TGCTGACGAG ATTCAACGGTC AGCAGAATGT CATCGTGGGT TCCAGGATCC 140
141 TCGAAGCTGT CTGATGAGTC CGTGAGGACG AAACCGCGTT GACAATTCCA AGGGTCTGGG CAACGACGAC 210
211 GATGAGGTAC CACATCGTGG TCGTTGCGCA CTGATGAGGC CGTGAGGCCG AAACCCCTGA CGCGTTCTTA 280
281 TGGGCGCGCT CTAGAGGGGC CAATTCGGCC TATAGTGGT CGLATTAACA TTCACTGGCC GTCGTTTTC 350
351 AACGTCTGTA CTGGGAAAAC CCGGGCGTTA CCCAACTTAA TCGGCTTGCA GCACATCCCC CTTCGCCAG 420
421 CTGGCGTAAT AGOGAAGAGG CCGGCACCGA TCGGCTTTC CAACAGTTGC GCAGGCTGAA TGGCGAATGG 490
491 ACGGCGCCCTG TAGCGGCGCA TTAAGCGCGG CCGGTTGGT GGTACGGGC AGGTGAGGCG CTACACTTGC 560
561 CAGGCGCCCTA GCGCGCGCTC CTTCGGCTTT CTTCCTTTC TTCTCGCA CGTTGCGCG CTTCCTCGT 630
631 CAAGCTCTAA ATCGGGGCT TCCTTTAGGG TTCCGATTTA GTGCTTTACG GNACTTACG CCAAAAAAC 700
701 TTGATTAGGG TGATGGTCA CGTAGTGGC CATTCGGCTG ATAGAACGGT TTTTGGGCC TTTTGACGTT 770
771 TGGAGTTCCA AGGTTCTTTT AATAGTGGC TTCTTTGGT CCCAACTGG GAACCAACCA CTMTAAACCC 840
841 TTATTTTGG GNCCTATTC CTMTTGGAA TTTAATTAG GGGAAATTTG GCGGATTTT TGGGGGCTT 910
911 TTTTGGGTTN AAAAAAATG GAGCTTGANT TTTAACCATA AAATTTTNA CCGCGGAAAN TTTTAAACCA 980
981 AAAANITINA GGGGGCCCCA ANGGGGCTTG NTTTAAAGGG GAAACCCGGG AACCCCTNT TAAAAAGCCC 1050
1051 AATTCCTC 1120
```

PCR II / chop - 43

Right!



Model 377
Version 2.1.1

11•GC424

Signal G:245 A:228 T:202 C:231
DT (BD Set Any-Primer)

Page 1 of 3
9:00 AM

Data Collection

File: 11•GC424
Sample: GC424
Comment:
Lane Number: 11
Channel Number: 62
Number of Scans: 12992
Length: 1057
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1280
Base Call End: 12992
Primer Peak Loc.: 1280
Signal: G (245), A (228), T (202), C (231)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.01 - ABI100

```
1  NCCAGCTATT TAGGTGACAC TATAGAATAC TCAAGCTATG CATCAAGCTT TGGAAACCCIG ATGAGTCCGT 70
71  GAGGAAGAAA CGATGACATT CTGCTGACCA GATTCAAGGT CAGCAGAATG TCATOGTCGG TTCCAGGATC 140
141 CTCTTCGACT GATGAGTCGG TGAGGACGAA ACATGGCTGA ATTCCAAGGG TCTGGGCAAC GACGACGATG 210
211 AGGTACCACA TGTGTGTGCT TCGGCACTGA TGAGGCGGTG AGGCCGAAAC CCTTGACGGG TTCCATATGG 280
281 GCGGCTCTAG AGGGCCCAAT TCGCCCTATA GTGAGTGTGA TTACAATICA CTGGCCGTGG TTTTACAACG 350
351 TGTGACTTGG GAAAACCCIG GCGTTACCCA ACTTAATCGC CTTCGAGCAC ATCCCCCTTT CGCCAGCTGG 420
421 CGTAATAGCG AAGAGGCCCG CACCGATCGC CCTTCCCAAC AGTTGCGCAG CCTGAATGGC GAATGGACGC 490
491 GCCCTGTAGC GCGCAATTAA GCGCGGCGGG TGIGGIGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC 560
561 GCCCTAGCGC CCGCTCCCTT CCGTTCTCTC CCTTCCCTTC TCGCCACGTT CCGCGGCTTT CCGCGTCAAG 630
631 CTCTAAATCG GGGGCTCCCT TTAGGGTTCC GATTTAGTGC TTTACGGGAC CTNGACCCA AAAAAGTTGA 700
701 TTAGGGTGAA TGGTTCAAGT AGINGGGCCA TTGCCCTTGA TANAAGGTTT TTTCGCCCTT TGGACCTTTG 770
771 GAAGTCCACG TTNTTTAAT AGNGGACNTT TTGGTTCCAA AACTGGNACC AACNANTINA ACCCTTATTT 840
841 TGGGTCTAAT TCTTTTGGCA ATTTATTAGG GCGATTTTGG GCGCGATTTT CCGGCCCTTT TGGGGTTNAA 910
911 AAAAAATGGA ACCTTGATTT TNACCCAAA AATTTTAAAN CCGCGAATTT TTTTAACCAA AAAATTTTANG 980
981 GGGCCCCAAA GGGGCTTTTG NTTTAAAGGG GAAACNGGG AACCCCTTTN AAAAAGGCCC TTTCCCNAA 1050
1051 AAAANCG 1120
```

PCR II / chop - C9 Right!



Model 377
Version 2.1.1

13-GC426

Signal G:288 A:270 T:244 C:265
DT (BD Set Any-Primer)

Page 1 of 3
9:00 AM

Data Collection

File: 13-GC426
Sample: GC426
Comment:
Lane Number: 13
Channel Number: 72
Number of Scans: 12992
Length: 1041
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1280
Base Call End: 12992
Primer Peak Loc.: 1280
Signal: G (288), A (270), T (244), C (265)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.02 ~ ABI100

```
1  CCAGCTATTTC AGGTGACACT ATAGANTACT CAAGCTATGC ATCAAGCTTTC GGAACCCCTGA TGAGTCCGTG 70
71  AGGACGAAAC GATGACATTC TGCTGACCAG ATTCAOGGTC AGCAGAATGT CATCGTCGGT TOCAGGATCC 140
141 TCCTGACTA ATGAGTCCGT GAGGACGAG CATGGCTGAA TTCCAAGGGT CTGGCGAAG ACGACGATGA 210
211 GGTACCACAT CGTCGTGCTT GCGCACTGAT GAGGCGGTGA GCGCGAAACC CTTCAGCGGT TCCTATGCGG 280
281 CCGCTCTAGA GGGCCCAATT CCGCCATATAG TGAGTCGTAT TACAATTCAC TGGCCGTGCT TTACAAACGT 350
351 CGTGACTGGG AAAACCCCTGG CGTTACCCAA CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCGAGCTGGC 420
421 GTAATAGCGA AGAGGCGCGC ACCGATCGCC CTTCCTCAACA GTTCGCGCAGC CTGAATGGCG AATGGAGCGG 490
491 CCTGTAGCG GCGCATTAAG CGCGCGGGT GTGGTGGTGA CCGCGAGCGT GACCGCTACA CTTCGCCAGCG 560
561 CCTAGCGCG CCGTCCTTTC GCTTCTCTCC CTTCCTTCT CCGCACGCTC GCGGCTTTC CCGTCAAGC 630
631 TCTAAATCGG GGGCTCCCTT TAGGGTTCG ATTTAGTCT TTACGGNACC TCGACCCCAA AAAACTTGAT 700
701 TAGGGTGATG GGTCAAGTAG TGGGCCATCG CCTTGATAG ACGGTTTTTC GGCCTTTGAC GTTGAAGIN 770
771 CAGGTCCTTC AATAGTGGAC TTCTTGGTTC CAAACTGGGA ACAACACTIN AACCTTATC TINGGCTAT 840
841 TCTTTTIGAA TTTATTTAAG GGAATTTTC CCGATTTTCC GGGCTATIN GGTINAAAA AAATGGAAGC 910
911 TTGANTTTTA ACCAAAAAT TTTTAACCGC GGAAATTTT AACCAAAAAN TTTCAGGGG CCGCAAGGGG 980
981 CTTCCTTTAA AGGGGAANCC GGAACNCC TTTINAAAAG GCGCAGINCC GCAANAAAAAN G 1050
```

PCR II / chop - C9 - m Right!



Model 377
Version 2.1.1

15-GC428

Signal G:316 A:288 T:245 C:276
DT (BD Set Any-Primer)

Page 1 of 3
9:01 AM

Data Collection

File: 15-GC428
Sample: GC428
Comment:
Lane Number: 15
Channel Number: 81
Number of Scans: 12992
Length: 1079
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1268
Base Call End: 12992
Primer Peak Loc.: 1268
Signal: G (316), A (288), T (245), C (276)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.01 - ABI100

```
1  CNCCAGCTAT TTAGGTGACA CTATAGAATA CTCAAGCTAT GCATCAAGCT TTGGAACCTT GATGAGTCCG 70
71  TGAGGACGAA ACGATGACAT TCTGCTGACC AGATTACCG TCAGCAGAAAT GTCATCGTGG GTTCCAGGAT 140
141  CCTTCAAAGA CTGATGAGTC CGTGAGGAGC AAACGAGGAT CGAATTCGAA GGGTCGCGC AACGACGACG 210
211  ATGAGGTACC ACATCGTGGT CGTTGCGCAC TGATGAGGCC GTGAGGCCGA AACCTTGAC GCGTTCTTAT 280
281  GCGGCGCTC TAGAGGCGCC AATTCGCGCT ATAGTGAGTC GTATTACAAT TCACITGGCG TCGTTTTTACA 350
351  ACGTCGTGAC TGGGAAAACC CTGGCGTTAC CCAACTTAAT CGCCTTGCG CACATCCCCC TTTCGCCAGC 420
421  TGGCGTAATA GCGAAGAGGC CCGCACCGAT CGGCTTTCC AACAGTTGCG CAGCCCGAAT GCGGAATGGA 490
491  CGCGCCCTGT AGCGGCGCAT TAAGCGCGGC GGGTGTGGTG GTTACGCGCA GCGTGACCGC TACACITGCC 560
561  AGCGCCCTAG CGCCCGCTCC TTTCGCTTTC TTCCCTTCTT TTCTCGCCAC GTTCGCGCGC TTTCGCCGTC 630
631  AAGCTCTAAA TCGGGGGCTC CCTTTAGGGT TCGATTTAA GTGCTTTACG GNACCTGAC CCCAAAAAAC 700
701  TTGATTAGGG TGATGGGTC ACGTAAAGG CCAATCGGCT GATAGACGGG TTTTTCGCGC TTTTGAAGTT 770
771  GGAAGTTCCA CGTTCTTTT AATAGTGGGA CTCTTTGGTT CAAAACCTTG GGAACCAACC ACTTTAAACC 840
841  CCTTATTTTT NGGCGCTAAT TCTTTTNGA ATTTAATTA GGGGAATTTT TGGCGAANT TTCNCGGCT 910
911  TATTTGGGGT TAAAAAATA TCGAANCTTG GATTTTNAAC CAAAAAAN TTTTAAACG CGGAAATTT 980
981  TTTAANCCAA AAATTTCAAN GGGGCCNCA ANGGGGCTT GGTTTNAAAG GGGAAACCG GNAACCCCT 1050
1051  TNNAAAAAGG NCCAATTNCC CCAAAAAA 1120
```

PCR II / Chop - Pol II Right!

Sample



Model 377
Version 2.1.1

05-GC418

Signal G:263 A:241 T:214 C:236
DT (BD Set Any-Primer)

Page 1 of 3
8:59 AM

Data Collection

File: 05-GC418
Sample: GC418
Comment:
Lane Number: 5
Channel Number: 36
Number of Scans: 12992
Length: 1091
Run started at: 15:25
Run stopped at: 02:26
Gel: Gel File
Dyeset/Primer: DT (BD Set Any-Primer)
Comb: 36-well sharks-tooth
Instrument Name: 377
Collect Vers.: N/A

Data Analysis

Base Call Start: 1265
Base Call End: 12992
Primer Peak Loc.: 1265
Signal: G (263), A (241), T (214), C (236)
Matrix Name: dRhodamine
Channels Ave.: 3
Analysis Vers.: Version 2.1.1
Base Spacing: 12.00 - ABI100

For

HindIII

BamHI

XbaI

1	CNCCAGCTAT	TTAGGTGACA	CTATAGAATA	CTCAAGCTAT	GCATCAAGCT	TTGGAAACCT	GATGAGTCCG	70
71	TGAGGACGAA	ACGATGACAT	TCTGCTGACC	AGATTACCGG	TCAGCAGAAT	GTCATCGTGG	GTTCCAGGAT	140
141	CTTGCTCTTC	TGATGAGTCC	GTCAGGACGA	AACCGCCTGA	GAATTCCAAG	GGTCTGCGCA	ACGACGACGA	210
211	TGAGGTACCA	CATCGTCGTC	GTTGCGCACT	GATGAGGCCG	TGAGGCGGAA	ACCCCTTGACG	CGTTCTATG	280
281	CGGCGCTCT	AGAGGGGCGA	ATTGCGCCTA	TAGTGAAGTGG	TATTTACAATT	CACCTGGCGGT	CGTTTACAA	350
351	CGTCGTGACT	GGGAAAACCC	TGGCGTTACC	CAACTTAATC	GCCTTGCAGC	ACATCCCCCT	TTCGOCAGCT	420
421	GGCGTAATAG	CGAAGAGGCC	CGCACCGATC	GCCCTTCCCA	ACAGTTGGGC	AGCCCTGAATG	GCGAATGGAC	490
491	GCGCCCTGTA	GCGGGGCATT	AAGCGCGGCG	GGTGTGGTGG	TTACGCGCAG	CGTGACCGCT	ACACTTGCCA	560
561	GCGCCCTAGC	GCCCGCTCCT	TTCGCTTTCT	TCCCTTCCCT	TCTCGCCACG	TTCGCGGCT	TTCGCGTCA	630
631	AGCTCTAAAT	CGGGGGCTCC	TTTAGGGTTC	CCGATTTAAT	GCTTTACGGG	ACCTTGACCC	CAAAAACTT	700
701	GATTAGGGTG	ATGGTTCACG	TAATGGGGCC	ATNGNCCITG	ATAGACGGTT	TTTTGNCTTT	TTGACGTTTG	770
771	GNAGINCAAC	GTTTCNTTIN	AATAGTGGGA	CCINTTGGIT	TCCCAACTT	GGGAACCAA	CAACTTTTAA	840
841	NCCCTTTTTC	TTTGGGGCCT	AATTCCTTTT	TGGANHTTTA	TINAAGGGGG	ATTTTTTGGC	CCGAATTTTC	910
911	NGGGCTTTT	TTGGGGTTAA	AAAAAAATGG	GAGCTTGGGA	TTTTTAACCA	AAAAANITTT	TAAACCGCGG	980
981	AAATTTTTTA	ACCAAAAAAT	TTTANGGGGC	NCCCAAAGGG	GGCTTTGNTT	TAAAGGGGAA	AACCGGGAAA	1050
1051	CCCCCTTTTA	AAAAGGGGCC	ATTTCCCCCN	AAAAAAACCN	G			1120

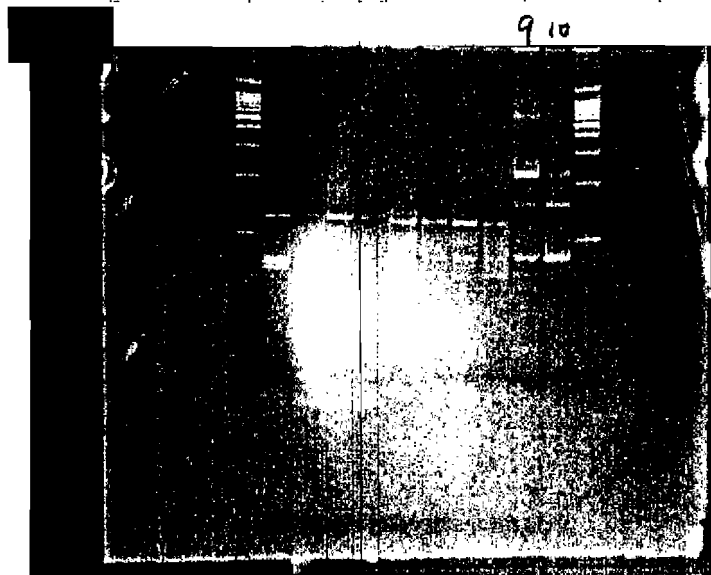
PCR II / chop - B2

right!

Chop #3 : HindII - BglII - StyI - XbaI.

[Redacted]

1	Ge	isolate	[Redacted]	PCR #1 +	[Redacted]	1. (Chop)
2	[Redacted]	PCR #2 +	[Redacted]	#4	(dG - chop)	
3		Primers	templet			
4	1.	413 + 414	> pNewclip			57°C Annealing
5	2.	413 + 415				
6	3.	413 + 414	> pChop #3			94°C 30"
7	4.	413 + 415				57°C 40"
8	5.	431 + 433	> pNewclip			72°C 1'
9	6.	432 + 433				
10	7.	431 + 433	> pChop #3			
11	8.	432 + 433				
12	9.	413 + 433	Chop temp			
13	10.	413 + 433	dG chop temp			



gel purify fusion bands 9 and 10



PCR screen condition:

10 p.m. 104. 1 M
 " 105 1 M.
 1 M 4 mM dNTP.
 Tag 14/x 1 M
 Buffer 2 M
 MgCl₂ 25 mM. 2 M.
 DNA 1 M (cells)
 H₂O 11 M

94°C 30"
 52°C 40"
 72°C 60"
 28 cycle.

Run 1.6% gel check:

Marker. 8x174 6 M. 1-14. per PCR.
 " 15-28 "

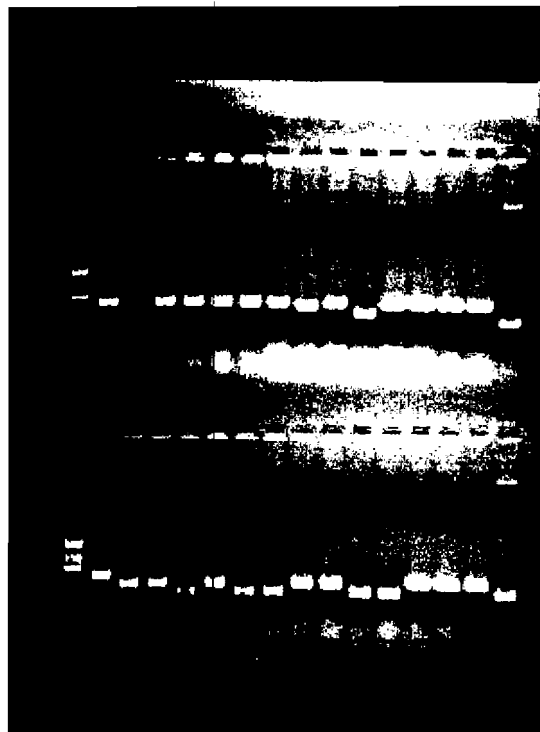


IMAGE SIZE (640 x 400 x 8).
 INT PERIOD = 0.46 SEC.
 ACQUIRED
 STRATAGENE EAGLE EYE II
 21:56:44

Chop:
1. 3. 4. 5. 6.

dG - Chop.
 7. 9. 11. 12.
 13. 14. 15.
 maybe right.
 Zuo er late to
 1 R + A. D

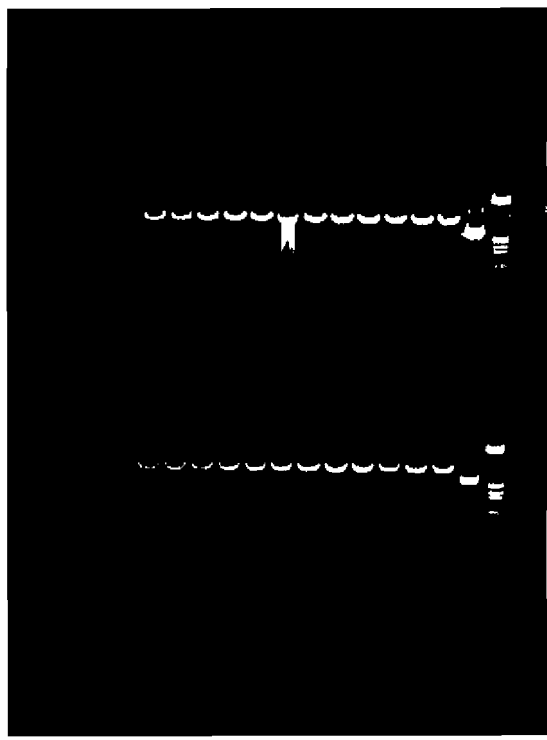
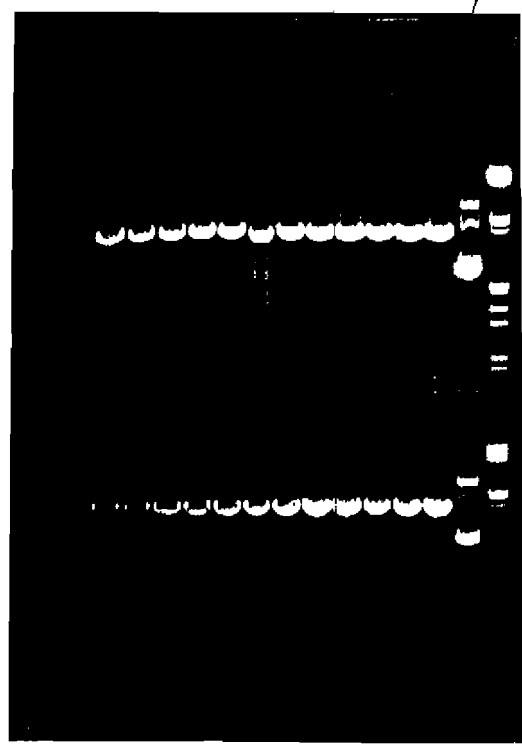


Isolate plasmid. Cut them with
BamH₁ or EcoR₁ old Chop #3 as
Control. (cut 3.5 ml)
Lane order:
BamH₁ 1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, old #3. Marker
EcoR₁ _____
#3. old.

841
HindIII @ EcoR₁

BamH₁

EcoR₁



STRATAGENE EXPOSURE 11
321:30:50
IMAGE SIZE: 400 x 400 x 81
DIT PERIOD: 0.73 SEC
ACQUIRED

old #3 is: HindIII - BglII - SfiI - XbaI. Can't be a.
all of the candidate can be cut. Sent to

Plan to Put B2 B2 mutant . C3 . C3 mutant . C9 .
 C9 mutant . pol I . pol I mutant to chop .
 B2 . C3 . C9 . pol I to dA-chop .

Anneal oligos :

1	Claw 439 + Claw 440	B2
2	" 441 + 442	B2 mutant
3	443 + 444	C3
4	445 + 446	C3 mutant
5	447 + 448	C9
6	449 + 450	C9 mutant
7	455 + 456	Pol I
8	457 + 458	Pol I mutant .

→ 1 μ m each in 50 μ l H₂O . 95°C 10 min . Continue
 reduce temperature to RT within 1h .

Put C3, C9 B2 pol2 to chop/PCR

Chop #4. EC387 is right! Finally!
Inoculate it to 25 ml LB + Amp

Make stocks: PCR/Chop. 1/98.

Isolate plasmid. 4 miniprep. Final
320 M. 0.2 µg/µl.

Cut with BamHI + EcoRI:

1. 30 µl DNA + 30 µl H₂O + 7 µl NEB2 1.5 µl BamHI
1.5 µl EcoRI

2. 20 µl sequencing left

1% SeaPlaque gel isolate GeneClean,
Final in 20 µl H₂O

Use 2 Ligation

			Buffer	Ligase
1	B2	1-10.5 µl + 2 µl V	1.2 µl	0.6 µl
2	B2-m	"	"	"
3	C3	"	"	"
4	C3-m	"	"	"
5	C9	"	"	"
6	C9-m	"	"	"
7	pol1	"	"	"
8	pol1-m	"	"	"
9	chop/PCR. HindIII/XbaI Vector only #2. 2 µl	"	"	"
	add H ₂ O to 12 µl 18°C o/n			



	1	2	3	4	5	6	7	8	9
--	---	---	---	---	---	---	---	---	---

1
2
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31



transform 5/7 ligation start to PHSA.



PCR Screen

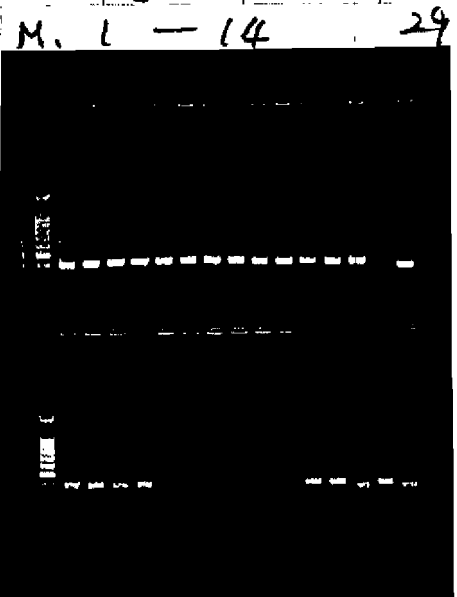
104
105
B2 : 1-6
B2-m : 7-12
C3 : 13-18
C3-m : 19-24 X

C9 : 25-28
C9-m : 31-36
POL2 : 37-39
POL2-m : 40-42

29, 30. PERE/chop V.

387, 388. PIND/chop : 47-56, 57 = PIND V.

Run gel.



#14 is wrong.
#19-24 all of the C3-m are wrong.
#27 is wrong.



	1	2	3	4	5	6	7	8	9
1	Screen the left.							PCR/Chop.	
2									
3	100bpM.	31-36.	V.	37-42.	V.				
4									
5	M. 100bp	47-57.		100bpM.					
6									

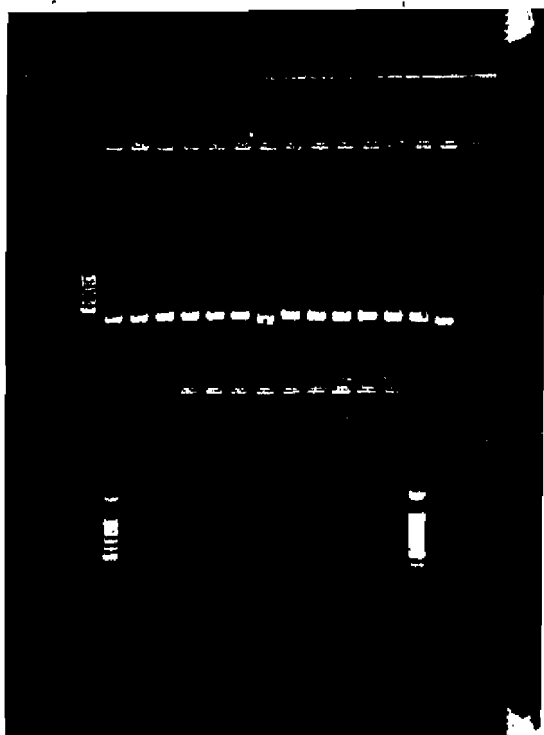


IMAGE SIZE (640 x 480 x 8),
2nd PERIOD = 2.49 SEC.
ACQUIRED
STORAGE ENGINE EYE II
17:27:26

22
23
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25
26
27
28
29
30
31

Inoculate these to 4ml LB + Amp:

B2 = 1, 2.

B2-m 27, 8.

C3: 13, 15.

C9: 25, 26.

C9-m: 31, 32.

pol2: 37, 38.

pol2-m: 40, 41.

Isolate plasmids

OD260:

according to Seq. res
Save

B2	#1	0.08232	GC 418
	2	0.09574	419
B2-m	7	0.08361	420
	8	0.09139	421
C3	13	0.11688	422
	15	0.11341	423
C9	25	0.09106	424
	26	0.11543	425
C9-m	31	0.09956	426
	32	0.10767	427
Pol 2	37	0.04975	428
Pol 2	38	0.11219	429
Pol 2-m	40	0.08922	430
	41	0.12112	GC 431

Sent to be sequenced using Clow 105

PIND/chop.	#50	0.09663	GC 432	✓
PIND/chop	#51	0.10231	GC 433	✓